CHAPTER 1

GENERAL INFORMATION

1.1 PURPOSE

This handbook establishes official procedures for determining aflatoxin in grain and processed grain products, and certifying the official results.

1.2 BACKGROUND

Aflatoxin is a naturally occurring mycotoxin produced by two types of mold: *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus* is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought. The mold occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Favorable conditions include high moisture content and high temperature. At least 13 different types of aflatoxin are produced in nature with aflatoxin B1 considered as the most toxic. While the presence of *Aspergillus flavus* does not always indicate harmful levels of aflatoxin it does mean that the potential for aflatoxin production is present.

GIPSA provides aflatoxin testing service as official criteria for corn, sorghum, wheat, and soybeans, as official criteria under the United States Grain Standards Act (USGSA). Testing is also provided for rice, popcorn, corn meal, corn gluten meal, corn/soy blend, and other processed products governed by the Agricultural Marketing Act (AMA).

Aflatoxin testing services are available nationwide, upon request and for a fee, as either a qualitative (screening above or below a threshold determined by the customer) or as a quantitative (actual results in parts per billion) service using several different types of test kits approved by GIPSA.

To further assist the grain industry, GIPSA also provides, on a limited basis, a complex chemical testing method, High Performance Liquid Chromatography (HPLC) testing for aflatoxin. The HPLC testing procedure is performed, upon request, for Board Appeal inspections only. All official aflatoxin testing is performed as prescribed in the GIPSA directive by authorized employees of GIPSA or licensed delegated/designated agency personnel.

Individuals wanting official aflatoxin testing should contact the nearest FGIS field office or delegated/designated agency.

1.3 MANDATORY TESTING

The 1990 Farm Bill (Food, Agriculture, Conservation, and Trade Act of 1990, P.L. 101-624) amended section 5 of the USGSA to "... require that all corn exported from the United States be tested to ascertain whether it exceeds acceptable level of aflatoxin contamination, unless the contract for export between the buyer and seller stipulates that aflatoxin testing shall not be conducted."

1.4 CONTAMINATION LIMITS

The Food and Drug Administration (FDA) has established action levels for aflatoxin present in food or feed. These limits are established by the Agency to provide an adequate margin of safety to protect human and animal health.

FGIS and FDA, having certain related objectives in carrying out their respective regulatory and service functions, have an agreement (Memorandum of Understanding) to assure the most effective possible system for identifying lots of grain, rice, pulses, and food products which exceed the FDA action levels of aflatoxin contamination. Under the provisions of the Memorandum of Understanding (MOU), FGIS and officially delegated/designated agencies report to FDA, on a lot-by-lot basis, each lot (grain, rice, and processed products) that, during the course of an official sample-lot inspection, exceed the 20 ppb FDA action limit.

Listed below are the FDA action levels for aflatoxins in animal feeds.

20 ppb	For corn and other grains intended for immature animals (including immature poultry) and for dairy animals, or when its destination is not known;
20 ppb	For animal feeds, other than corn or cottonseed meal;
100 ppb	For corn and other grains intended for breeding beef cattle, breeding swine, or mature poultry;
200 ppb	For corn and other grains intended for finishing swine of 100 pounds or greater;
300 ppb	For corn and other grains intended for finishing (i.e., feedlot) beef cattle and for cottonseed meal intended for beef cattle, swine or poultry.

Aflatoxin-contaminated corn lots may be reconditioned under the certain conditions established by FDA. (See Attachment)

1.5 APPROVED TEST METHODS

FGIS has approved test kits for use at field testing locations. The AflaCup, EZ-Screen, and Agri-Screen test kits are approved for qualitative analysis of corn. The Aflatest, Fluoroquant, Veratox-AST, and Myco✓test kits provide quantitative analysis but can be used for qualitative results. High Performance Liquid Chromatography (HPLC) testing is reserved for quantitative testing at the Technical Services Division (TSD) only.

The methods listed below have been conformance tested to perform within FGIS specifications. Each of the approved test methods has been certified to provide results accurate up to the conformance test level at which they were approved.

FGIS APPROVED TEST METHODS			
Method and Test Kit	Approved for		Conformance Limit(s)
	Qualitative	Quantitative	
AflaCup (International Diagnostics Inc.)	X		20 ppb
AgriScreen - (Neogen)	X		20 ppb
Veratox AST - (Neogen)	X	X	300 ppb (quantitative)
Fluoroquant - (Romer)	X	X	300 ppb (quantitative)
Aflatest	X	X	300 ppb (quantitative)
Myco✓ - (Strategic Diagnostics Inc.)	X	X	300 ppb (quantitative)

Listed in the table below are the test kits that are commonly used for official aflatoxin analysis. Use the table to determine the appropriate test kit(s) to use for testing the listed grain/commodity. For information concerning the testing of mixed grain, contact the Policies and Procedures Branch.

GRAIN/ COMMODITY	TEST METHOD					
	AflaCup	Aflatest	Agri-Screen	Fluoroquant	Veratox-AST	Myco✓
Corn	X	X	X	X	X	X
Sorghum		X		X	X	X
Wheat		X		X	X	
Soybeans		X		X	X	
Corn Screenings		(✻)			(✻)	
Corn Meal		X		X	X	X
Corn Germ Meal		X			X	
Corn Gluten Meal		X			X	
Corn/Soy Blend		X		X	X	X
Corn Gluten Feed		X				
Flaking Corn Grits		X		(✻)	(✻)	
Corn Flour					(✻)	
Corn Bran					(✻)	
Popcorn		X		X	X	X
Milled Rice		X		X	X	
Rough Rice					(✻)	
Cracked Corn	(✻)	(✻)	(✻)	(✻)	(✻)	(✻)

NOTE: An X entered into a block denotes that the test kit has been evaluated and approved for the grain/commodity.

The symbol (✻) entered into a block denotes that the test kit is under evaluation by TSD for the grain/commodity and is temporarily approved for official use.

1.6 DISCLAIMER CLAUSE

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

1.7 TESTING SERVICES

Applicants requesting aflatoxin testing must specify whether qualitative or quantitative testing service is desired. If qualitative analysis is requested, the applicant must specify the level desired (e.g., 20 ppb). Three types of aflatoxin testing services are available as follows:

a. Submitted Sample Service.

Analysis based on a sample submitted by the applicant for service.

b. Official Sample-Lot Service.

Analysis based on an official sample obtained and analyzed by official personnel.

(1) Single lot inspection.

Samples may be obtained and tested on either an individual carrier basis or a composite sample basis (maximum of five railcars or fifteen trucks per composite sample).

(2) Unit train inspection under the CuSum Loading Plan.

Unit trains are analyzed on a sublot basis for corn and sorghum and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

When aflatoxin testing is required, samples may be obtained and tested on either an individual carrier basis or a sublot basis. The maximum size sublot for aflatoxin testing is five railcars for unit trains consisting of less than 200,000 bushels, or less than fifty railcars. For unit trains consisting of 200,000 bushels or more, or fifty railcars or more, the maximum sublot size is ten railcars.

(3) Export shiplots

Export shiplots are analyzed on a sublot basis for corn and sorghum and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

(4) Supplemental Testing.

Upon request, supplemental testing may be performed as follows:

Composite samples may be analyzed in addition to the sublot test for corn and sorghum shiplots or unit trains.

(5) Alternate Testing.

Upon request, alternate testing methods may be used, provided that the minimum testing requirements are met. Examples of alternate testing are as follows:

(a) Sublot testing may be used instead of composite sample analysis for grains routinely tested on a composite basis.

(b) Grain shipments may be tested on a component sample basis in lieu of the sublot basis under the provisions of Book III, Inspection Procedures. Components are combined and averaged to determine the sublot result. Acceptable quality will be based on the sublot result as compared to the contracted "maximum" specification.

c. Warehouse Sample-Lot Inspection Service.

Analysis based on an official sample obtained by a licensed warehouse sampler and analyzed by official personnel.

1.8 REVIEW INSPECTIONS

Sections 800.125 and 800.135 of the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factor and official criteria may be handled separately even though both sets of results are reported on the same certificate.

Review inspection services for aflatoxin are provided on either a new sample or the file sample in accordance with the regulations. Board appeal inspection services are limited to the analysis of file samples.

Only one field review (reinspection or appeal inspection) is permitted for shiplot, unit train, or lash barge material portions when testing is performed on a sublot basis. However, if the applicant requests a review of the entire lot, up to three review levels of service (reinspection, appeal, board appeal) may be obtained for each sublot included in the lot. Inspection results for each review level shall replace the previous inspection result.

a. Reinspection Service.

The laboratory providing original testing services also provides reinspection services. Applicants may request either qualitative or quantitative analysis unless the original test was quantitative. Then, only a quantitative analysis is available.

b. Appeal Inspection Service.

FGIS field offices provide appeal testing services for aflatoxin. Field offices not equipped to provide testing will make arrangements with another FGIS office to provide the most timely service possible. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available. If samples are sent to a field office for analysis, write the words **"AFLATOXIN APPEAL"** in the “Remarks” section of the grain sample ticket and on the back of the mailing tag.

c. Board Appeal Inspection Services.

Board appeal inspection services are limited to the file sample and are provided by the Board of Appeals and Review (BAR) in Kansas City. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available. The HPLC method is also available for determining aflatoxin in Board appeal samples. The applicant must specify the HPLC method as the desired determination method. Otherwise, the Board appeal inspection will be conducted using the rapid method (test kits).

When sending samples to the BAR, write the words **"AFLATOXIN BOARD APPEAL"** in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

1.9 QUALITY ASSURANCE PROGRAM

The Technical Services Division (TSD), located at the Kansas City Technical Center, conducts an aflatoxin check sample program for all specified service points and laboratories providing testing services. TSD is responsible for preparing and distributing check samples each quarter to all official aflatoxin testing locations, analyzing check sample results, notifying field locations of any results indicating problems, and releasing a quarterly summary report to all participating laboratories. Field offices are responsible for routine supervision to assure all laboratories in their circuit provide accurate results. The TSD check sample program is designed to test the capability of the official system and to monitor the accuracy of approved testing methods. The check sample program provides limited performance information that can be used to supplement the routine supervision of official personnel performing testing services.

AFLATOXIN SAMPLING AND RECONDITIONING PROCEDURES

1. FDA RECONDITIONING GUIDELINES

The Food and Drug Administration (FDA) will permit reconditioning of aflatoxin-contaminated corn lots at export locations by mechanical cleaning under the following conditions:

a. Only one attempt at reconditioning is allowed. The analytical results from the reconditioned lot will be the final determination for disposition of the entire lot.

b. To assure proper reconditioning, the grain company must mechanically clean the lot at a rate not to exceed 50 percent of the rated cleaner capacity.

c. FGIS must oversee the cleaning process, sample the reconditioned lot (cleaned corn) using a diverter-type mechanical sampler, and analyze the samples for aflatoxin.

d. FGIS must sample the cleanings/screenings using the most practical procedures available and test the cleanings and/or screenings for aflatoxin contamination.

At interior locations, the local FDA office may modify the reconditioning procedures to provide for a cost effective process.

2. FGIS RESPONSIBILITIES

When positive lots are identified at export locations, field office managers (FOM) should work with the grain facility representatives and develop a standard operating procedure (SOP) for reconditioning aflatoxin-contaminated corn.

FOM's should review the SOP with local FDA officials before implementing the reconditioning process, unless instructed otherwise by FDA.

a. Export Locations

At export locations, FGIS or official delegated state agency personnel, as applicable, are responsible for:

(1) Reporting actionable lots to the local FDA field office.

(2) Preserving the identity of actionable lots prior to reconditioning.

(3) Monitoring the reconditioning process at the grain facility.

(4) Sampling and testing reconditioned lots (cleaned corn and screenings) for aflatoxin. When sampling screenings, use the most practical method available to obtain a representative sample.

(5) Preserving the identity of reconditioned lots and screenings. (Screenings are not considered a reconditioned lot.)

(6) Reporting aflatoxin results of reconditioned lots and screenings to FDA.

(7) Completing a report of the reconditioning process. Include in the report the following information:

(a) Date Reconditioned.

(b) Grain Elevator/Location.

(c) Type of Sample/Carrier.

(d) Original Results.

(e) Reconditioned Whole Grain Results.

(f) Cleanings/Screenings Results.

(g) Size of Cleaner Screens used to Recondition the Lot.

(h) Elevator Set-up Information.

b. Domestic Locations

FOM's servicing interior locations should contact the local FDA office servicing the area where the contaminated lot is located to discuss and determine responsibilities for managing the reconditioning process. Official agencies and affected grain companies are encouraged to participate in these discussions to facilitate the development of an SOP.

3. SAMPLE SIZE AND PREPARATION

Obtain the minimum sample size as directed in chapter 2 of this handbook. If requested by the applicant, a larger sample size may be obtained.

Grind the entire corn sample obtained for aflatoxin testing and prepare three 500-gram subportions from the ground sample.

Sample Portion	Use
Test Portion	Original inspection service
File Portion	Review inpection service
FDA Portion	Retain for FDA analysis if results exceed 20 ppb.

When reconditioned lots are resampled in accordance with the FDA guidelines, a file portion is not required.

If FGIS's original results for a reconditioned lot of corn or screenings exceed 20 ppb, the FDA sample portion will be used for any subsequent verification (by FDA) of results.

4. DISPOSITION POLICY

The grain industry must comply with FDA policy regarding the disposition of corn and screenings resulting from the reconditioning process. In general, disposition will occur as follows:

- Cleanings/screenings may be used for animal feed if the aflatoxin content meets FDA feed guidelines. The screenings may not re-enter food channels in any fashion.

- Reconditioned (cleaned) corn with less than 20 ppb aflatoxin may be handled without restrictions. When the reconditioning process fails and the corn continues to exceed the 20 ppb level, disposition is based on current FDA policy.

Contact the local FDA office regarding other questions concerning specific disposition action.